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Life cycle of *Puccinia acroptili* on *Rhaponticum* (= *Acroptilon*) *repens*

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Abstract: Russian knapweed (*Rhaponticum repens*) is a rangeland weed pest in the western United States. One candidate fungus for biological control of *R. repens* is *Puccinia acroptili*, which causes a rust disease. Understanding the life cycle of candidate rust fungi for weed biological control is an essential component in risk assessments and evaluations, and for *P. acroptili* such was unknown. For this reason greenhouse studies were undertaken to clarify the life cycle of *P. acroptili* under artificial conditions. Spermogonia with spermatia developed on *R. repens* following plant inoculation with teliospores. Artificial transfer of spermatia between spermogonia resulted in the development of aecia with uredinioid aeciospores. Inoculation with aeciospores or urediniospores resulted in uredinia containing urediniospores and occasional amphispores. Telia with teliospores and occasional mesospores developed later. Teliospores produced typical basidia with four basidiospores. These results suggest that the life cycle of *P. acroptili* is macrocyclic and autoecious. Inoculation with teliospores also frequently resulted in production of sori that were morphologically similar to aecia but which were not associated with spermogonia or the classical transfer of spermatia. The ontology of these sori is unknown. This is the first description of spermogonia and the first report and description of basidiospores, aecia, aeciospores, amphispores and mesospores of *P. acroptili*.

Key words: biological control, Cardueae, knapweed, Pucciniales

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INTRODUCTION

Russian knapweed, *Rhaponticum repens* (L.) Hildago (Hildago et al 2006; = *Acroptilon repens* (L) DC; Asteraceae), is a perennial weed introduced into North America from central Asia. It now is reported from the Pacific Coast of North America east to Ontario, Canada, and in continental USA to Ohio, Kentucky, Arkansas and Texas (USDA NRCS 2008). It is listed as a pest in 18 states, including Alaska and Hawaii, and infests pastures, rangelands and other dry habitats (DiTomaso and Healy 2007). Because of its widespread distribution and the difficulties in management with conventional control strategies *R. repens* has been subject of research about the potential for biological control (Bruckart et al 2005, Kim and Mortensen 1986, Mortensen et al 1991).

Puccinia acroptili Syd. & P. Syd. is a rust fungus (Pucciniales) that parasitizes *R. repens* in its native range in Eurasia. It is widespread also in North America (Bruckart et al 2006, Cummins 1979, Dugan and Carris 1992, Mortensen and Molloy 1989, Palm and Vesper 1991, Savile 1970b). Based on two-dimensional polypeptide mapping *P. acroptili* is distinct from other similar rusts on Cardueae in North America, including *P. jaceae* Otth and *P. centaureae* DC. on *Centaurea* spp. and *P. carthami* Corda on safflower (Kim and Mortensen 1986). An aggressive isolate of *P. acroptili* from Turkey was evaluated recently as a candidate for biological control of *R. repens* (Bruckart et al 2005), and comparisons with North American and other Eurasian isolates were initiated.

The life cycle of *P. acroptili* has not been documented, but it is generally considered autoecious. Spermogonia were observed only recently in the field (Mortensen and Molloy 1989), but they were not described. Savile (1970b) noted that "pycnia are unknown"; Cummins (1979) stated that "pycnia and aecia are unknown"; and Sydow and Sydow (1904) and Wei and Wang (1986) did not mention spermogonia in their descriptions. Aecia have not been reported or described, and there is no report of attempted crosses or functionality of different spore types, with the exception of urediniospores (Mortensen et al 1991).

Knowledge of the life cycle of candidate organisms is imperative for identifying sound biological control agents. Thus the objective of this study was to determine the life cycle of *P. acroptili* based on artificial inoculations under controlled greenhouse

TABLE I. Isolates of *Puccinia acroptili* examined in this study

Isolate[a]	Collector	Location	Notes		
			Latitude	Longitude	BPI No.
90-114	D.C. Sands	KAZAKHSTAN: Kurday			
01-595	B. Tunali	TURKEY: Beypazari			87 8931[c]
02-048	D.K. Berner	TURKEY: Ankara-Cankiri	39°55′43″	33°20′29″	87 7990[b], 87 7991[c]
05-051	M. Becktell	USA: Colorado, Skipper's Island	39°09′57″	−108°46′50″	
05-052	M. Becktell	USA: Colorado, Skipper's Island	39°09′59″	−108°46′54″	
05-053	M. Becktell	USA: Colorado, Skipper's Island	39°09′57″	−108°46′59″	
05-054	M. Becktell	USA: Colorado, Skipper's Island	39°09′47″	−108°46′53″	
05-055	J. Littlefield	USA: Bell Bottom, MT			87 8932[c]
05-056	J. Littlefield	USA: Freemont County, WY			87 8933[c]
05-069	S.G. Vesper	USA: Albuquerque, NM			111 0177[b]
05-070	S.G. Vesper	USA: Sandoval Co., NM			110 7952[b]
05-085	D.K. Berner	RUSSIA: near Taman, Mawritansky Lake	45°14′03″	36°46′27″	
06-037	M. Becktell	USA: Colorado, Skipper's Island	39°09′47″	−108°46′47″	87 8934[c]

[a] Foreign Disease-Weed Science Research Unit number.
[b] Field collection. Otherwise, spores were produced under artificial greenhouse conditions.
[c] Sample includes stages O and I. Note: All samples include stages II and III.

conditions. All sori and spore types produced by *P. acroptili* are described and illustrated.

MATERIALS AND METHODS

Isolates.—Eleven isolates of *P. acroptili* were received from Eurasia and USA as viable urediniospores and teliospores on dried leaves (TABLE I). A portion of urediniospores from each original sample was stored in freezer at −80 C. Urediniospores from original samples also were used for spore increase through artificial inoculation, and greenhouse-produced urediniospores were stored either at −80 C or 4 C. All teliospores, whether from original samples or from greenhouse production, were stored on dried-leaf material, either under refrigeration, some at very low temperatures.

Inoculations.—Inoculations were made most frequently with urediniospores or teliospores, but inoculation either with aeciospores or spores that developed in aecium-like sori after teliospore inoculations was made in a few cases to confirm viability and pathogenicity. Spores were suspended in distilled water plus a wetting agent (0.15% v/v Tween® 20 [polyoxyethylene sorbitan monolaurate]) and sprayed onto 4 wk old potted *R. repens* plants at 0.5 mg/plant. Plants were given two 16 h dew treatments at 18 C in the dark, with 8 h daylight between dew periods. Inoculated plants were removed from the dew chamber, benched in a containment greenhouse at 21–25 C with supplemental lighting to maintain a 14 h photoperiod and observed for infection.

Teliospores for inoculation were primed 1 wk by placing stems and leaves with telia in a Petri dish containing moist towels and incubating them under refrigeration. After priming ungerminated teliospores were suspended in water with wetting agent, as described, and applied either by finger to individual leaves or by spraying the teliospore suspension onto plants. Inoculated plants were incubated in a dew chamber at 18 C for 72 h with 16 h dark followed by constant light, thus triggering development of basidia and basidiospores on the leaves. Plants were placed on a bench in a cubicle in the containment greenhouse at 20 C with supplemental lighting as described. Plants were observed for symptoms, and data on sorus type and quantity were collected.

Crosses.—Leaves were inoculated with teliospores to produce spermogonia and spermatia for crossing experiments. When flecks appeared leaves were detached and leaf bases inserted through Parafilm® (Pechiney Plastic Packaging, Menasha, Wisconsin) into jars of sterile tap water (FIG. 1). Jars were put in insect exclusion cages to prevent uncontrolled crossing between spermogonia by shore flies (*Scatella stagnalis* [Fallen]) and fungus gnats (*Lycoriella* spp. and *Bradysia* spp.). Self-crosses were made with six of the isolates that included both USA and Eurasian accessions. Spermatia from each isolate were collected by excising a single, isolated, orange spermogonium from a leaf, washing it in 50 µL sterile distilled water and transferring 1 µL drops suspension to each of the remaining spermogonia of the same isolate. Transfers were made with a P2 Gilson/Rainin Pipeteman® (Gilson Inc., Middleton, Wisconsin) pipette with a new tip for each spermogonial transfer. Leaves were mapped for distribution of spermogonia. Jars with treated leaves were returned to insect exclusion cages as described, and leaves were observed for development of aecia. Spermogonia with aecia were recorded on leaf maps, and percentage of successful crosses was calculated for each isolate.

Measurements.—Spores were measured with a Nikon Eclips 80i microscope equipped with a DS-L1 camera and a flat-screen monitor. Software enabled on-monitor measurement of objects in the field of view, calibrated for each level of magnification. A minimum of 50 spores was measured for each isolate and spore form. In addition urediniospores and

FIG. 1. Detached leaves of *Rhaponticum repens* inserted through Parafilm® into flasks of sterile tap water and placed in an insect-proof cage.

FIG. 2. Leaf with uredinia, left, and leaf with orange clusters of spermogonia, right, or dark brown aecium-like sori that have developed without an observed fertilization event (i.e. transfer of spermatia or other visible sexual process).

teliospores were measured from two herbarium specimens collected in New Mexico and borrowed from the U.S. National Fungus Collections in Beltsville, Maryland (BPI 1107952 and 1110177, TABLE I).

Spores were mounted on slides with a drop of 1% aniline blue in lactophenol, and each slide was heated gently until spores were turgid (Savile 1970a). Urediniospores, aeciospores, and spores developing in the aecium-like sori following teliospore inoculations were measured both for length and width, and the average of these measurements was used for data analysis. Teliospores and spermatia were measured for length and width, and data for each of these dimensions were used directly in statistical analyses. Cross-sections of spermogonia, stained with lactophenol and aniline blue, also were measured as described.

Statistical treatments.—Calculation of means and confidence intervals ($P = 0.05$) were made with Microsoft® Office Excel 2003 software. Data either were pooled for all isolates or analyzed as the mean of isolate means. Results are presented as mean (± confidence interval).

RESULTS

All spore types were observed on leaves and stems of inoculated Russian knapweed plants except basidiospores, which were seen during microscopic examination of germinating teliospores on water agar. All tested spore forms were functional, resulting in completion of the life cycle of *P. acroptili* under greenhouse conditions.

Urediniospore inoculations.—Inoculations with urediniospores resulted in uredinia typical for *Puccinia* species on plants in tribe Cardueae (FIG. 2, left). Sori were brown with no associated hypertrophic leaf tissue. Urediniospores were golden brown and generally oval, had three more-or-less equatorial germ pores and were 22.1(± 0.4) µm diam (n = 10 isolates, TABLE II).

Telia and teliospores.—Telia developed eventually from urediniospore inoculations. Sori were dark brown with no surrounding hypertrophic leaf tissue. Teliospores were typically dark brown, 1-septate, with a distinct pedicel (FIG. 3). The average width × length for teliospores from 12 isolates was 23.1(± 0.4) × 38.1(± 0.8) µm (TABLE II). Germinating telio-

TABLE II. Dimensions (µm) of *Puccinia acroptili* spores from different sources (n ≥ 50 for each isolate)

	Source of spores		
	Telia		
Isolate	Width	Length	Uredinia[a]
90-114	22.2	39.3	22.4
01-595	23.0	38.5	22.7
02-048	22.0	41.2	22.4
05-051	22.7	38.0	22.5
05-052	23.6	38.1	21.6
05-053	23.3	37.3	22.1
05-054	24.0	37.5	nd
05-055	22.2	38.1	21.2
05-056	23.4	36.8	21.8
05-069	23.5	36.6	21.4
05-070	23.0	35.9	23.3
05-085	24.7	39.7	nd
Mean (ci[b])	23.1(0.4)	38.1(0.8)	22.1(0.4)

[a] Average of width and length.

[b] ci = confidence interval for the mean of the means ($P = 0.05$).

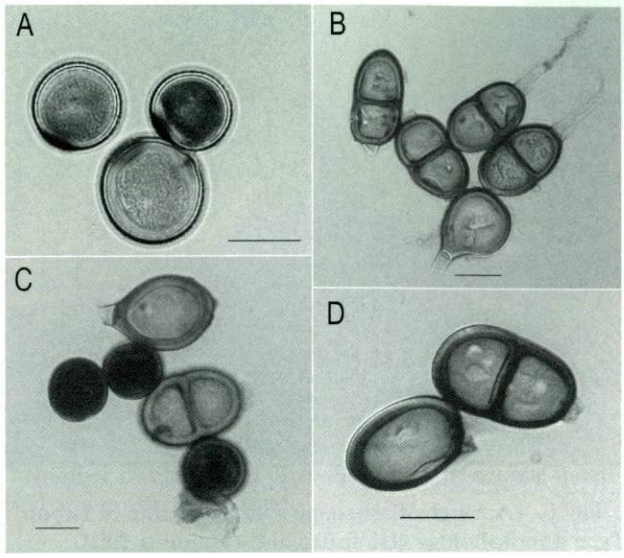

FIG. 3. A. Single amphispore, bottom, with two urediniospores. B–D. Teliospores and mesospores of *Puccinia acroptili* (after Kirk et al 2001). C. Three urediniospores present. Bar = 20 μm.

spores produced basidia with four basidiospores per basidium on water agar.

Amphispores and Mesospores.—Amphispores (FIG. 3A) and mesospores (FIG. 3B–D) were noted infrequently in greenhouse samples of urediniospores and teliospores respectively. Amphispores were similar in color and morphology to urediniospores, but they were significantly larger and had thick walls (Kirk et al 2001). Amphispores were 28.7(± 0.7) μm diam (n = 13). Mesospores were 1-celled teliospores produced among 2-celled teliospores (Kirk et al 2001), and they were 24.6(± 1.6) × 32.1(± 1.7) μm (n = 11).

Teliospore inoculations.—Inoculations with primed, ungerminated teliospores placed directly on plant leaves resulted in development of two types of sori, yellow-orange spermogonial sori and aecium-like sori (FIG. 2, right). Orange aggregations of spermogonia were evidence of basidiospore infections and appeared on hypertrophic leaf tissue surrounding the sori. However aecium-like sori developed in greater proportion than spermogonia after teliospore inoculations (FIG. 2, right). Aecium-like sori were produced by all six isolates tested, and they occurred 1.8 times more frequently than spermogonia for three isolates, 02-048, 05-085 and 05-055, for which data were collected. These sori, which were surrounded by hypertrophic leaf tissue, were not associated with spermogonia.

Spores from aecium-like sori.—Aecium-like sori resulted directly from teliospore inoculations and generated spores that were morphologically similar to aeciospores. Mean diameter of spores from aecium-like sori of three isolates (TABLE III) was 24.8(± 1.1), which is significantly larger than those of normal urediniospores (TABLE II) but not different from aeciospores (TABLE III).

Spermogonia and spermatia.—Spermogonia generally were aggregated into yellow-orange sori (FIG. 4) that were covered with a sugary matrix of spermatia. Individual spermogonia were flask-shape with flexuous hyphae extending from an ostiole (FIGS. 5, 6). For isolate 02-048 spermogonia were 127.5 ± 10.9 μm diam (n = 25) and were classified as typical *Puccinia* Group V, Type 4 spermogonia (Hiratsuka and Cummins 1963). Spermatia were oval, hyaline and small at 2.1(± 0.1) × 3.3(± 0.2) μm wide and long respectively for isolates 02-048 and 05-055 (TABLE III).

Fertilization of spermogonia; aecia and aeciospores.—Aecia developed from self crosses within (each of the six isolates (TABLE IV, FIGS. 4, 6). No insect activity was detected inside cages, so both successful and unsuccessful crosses resulted from the artificial transfer of spermatia. Aecia were similar to uredinia,

TABLE III. Dimensions (μm) of spores from spermogonia, aecium-like (AL) sori[a], aecia, and dimensions of urediniospores following inoculation by spores from either AL sori or aecia (n ≥ 50 for each isolate)

	Source of spores			Urediniospores from inoculation by spores from:	
Isolate	Spermogonium	AL[a]	Aecium	AL	Aecium
02-048	2.1 × 3.1	24.0	23.6	22.2	21.4
05-055	2.2 × 3.3	24.4	23.7	23.3	22.5
05-085	nd[b]	nd	25.3	22.8	23.4
06-037	nd	26.1	25.2	nd	22.3
Mean (ci[c])	(see text)	24.8(1.1)	24.5(0.9)	22.8(0.5)	22.4(0.8)

[a] Sori from teliospore inoculations that are aecium-like and not the result of a cross between compatible spermogonia.
[b] nd = not determined.
[c] ci = confidence interval for the mean of the means ($P = 0.05$).

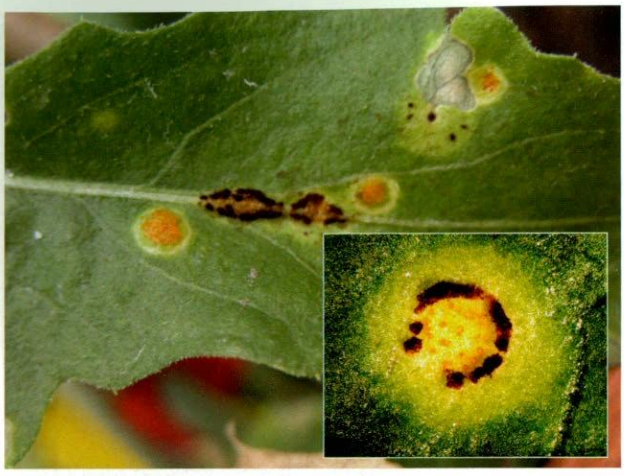

FIG. 4. Four clusters of spermogonia, each inoculated with spermatia from a single sorus. The two orange sori on either end were not fertilized and aecia did not develop after the transfer of spermatia. Two sori between the orange unfertilized sori are ringed by aecia, results of compatible crosses. Insert: A cluster of spermogonia with a ring of aecia.

but they either developed within or on the margins of spermogonial clusters (FIG. 4) or they emerged from the opposite surface of the leaf from spermogonia (FIG. 6). Aeciospores were also similar to urediniospores (i.e. uredinioid aeciospores) in gross morphology, but they, like spores from the aecium-like sori, were significantly larger (TABLE II) than urediniospores (TABLE II). Mean aeciospore diameter from four isolates was 24.5(± 0.9) (TABLE III).

Plant inoculations either with aeciospores or spores from aecium-like sori.—Inoculations using either of these spores resulted in development of uredinia (i.e. sori with no associated hypertrophic growth and producing the dikaryotic repetitive spore stage). Urediniospores from these sori were the same size (TABLE III) as other urediniospores in the study (TABLE II).

DISCUSSION

Results of these investigations indicate that *P. acroptili* is autoecious and macrocyclic. All known spore forms of *Puccinia* were observed and found functional. Sizes of both urediniospores and teliospores were within the ranges described by others for *P. acroptili* (Bruckart et al 2006, Cummins 1979, Dugan and Carris 1992, Mortensen and Molloy 1989, Palm and Vesper 1991, Savile 1970b, Sydow and Sydow 1904, Wei and Wang 1986). Basidiospores, spermogonia and spermatia, aecia and aeciospores, amphispores and mesospores had not been described before this study, although spermogonia were reported by Mortensen and Molloy (1989) from field observations.

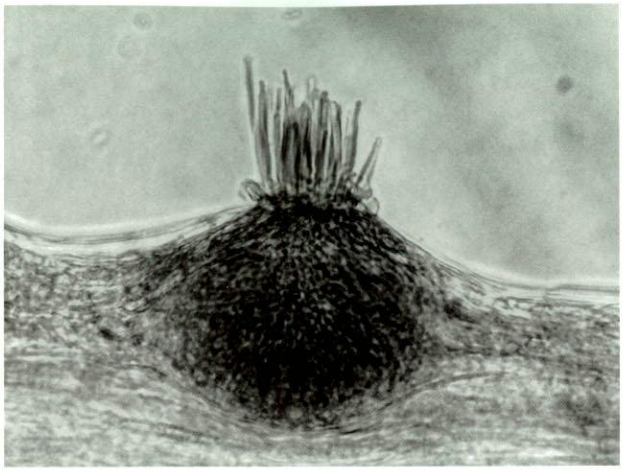

FIG. 5. A. Single, flask-shape spermogonium of Group V Type 4 morphology (Hiratsuka and Cummins 1963).

Production of aecium-like sori directly from inoculation with teliospores was not expected and is reported herein also for the first time. These sori were similar to aecia in color and symptomatology, including hypertropic growth of plant tissue surrounding sori, but they were not associated with spermogonia or derived from any observed classical fertilization. Position in the life cycle (i.e. development following teliospore inoculations) and the associated hypertrophic leaf tissue distinguished them from uredinia. Spores from these sori were the same size and shape as those produced in aecia, and when these were inoculated onto plants uredinia developed as would be expected after inoculation by aeciospores. Ontogeny and karyogamy of the spores produced in aecium-like sori is not known.

At least two examples have been reported of aecial

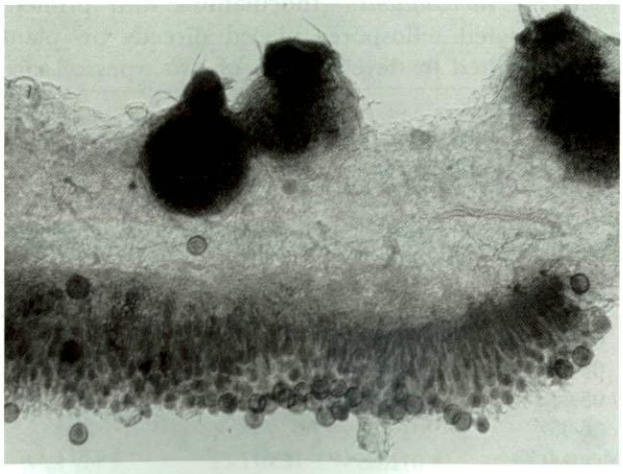

FIG. 6. Cross section of a leaf showing three spermogonia on the upper (adaxial) surface and an aecium on the lower (abaxial) surface.

TABLE IV. Percentage success for crosses within isolates (self crosses) of *Puccinia acroptili* from different source locations

		× Self	
Isolate	Location	Success[a]	Attempts[b]
02-048	Turkey	15	3
05-085	Russia	48	5
90-114	Kazakhstan	33	1
05-055	Montana	31	4
05-056	Wyoming	26	2
06-037	Colorado	42	3
Mean		32.5	3.0

[a] Percentage of spermogonia that developed aecia.

[b] Number of times a set of *Rhaponticum repens* was inoculated and crosses attempted.

development without pycnia. These concern *Puccinia allii* (DC) Rud. (Anikster et al 2004) and *P. coronata* Corda f. sp. *bromi sensu* Mühlethaler (Anikster et al 2003). In each of these cases the variant of the fungus produced only two spores per basidium and the nuclear condition of each basidiospore was double that of typical basidiospores. These two cases differ from that observed for *P. acroptili*, in which four basidiospores were observed per basidium. Investigation is under way into leaf surface germination and infection after teliospore inoculation with *P. acroptili*.

Amphispores and mesospores were seen only rarely. No tests were made with these spore forms because they were uncommon, occurred in mixtures with other spore types and generally comprised only a small proportion of spores in a sample. Amphispores or mesospores have not been reported previously in the literature for this rust, and it is not known whether they are produced under natural conditions.

In summary we report the production of eight different spore types by *P. acroptili*: teliospores, mesospores, basidiospores, spermatia, aeciospores, spores produced in aecium-like sori, urediniospores and amphispores. In addition to providing detail about spore forms and their functionality data from this study suggest that *P. acroptili* is autoecious. The autoecious nature of this fungus is important evidence that *P. acroptili* is host specific, a character that is essential for plant pathogens under consideration for weed biological control in North America (Berner and Bruckart 2005).

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LITERATURE CITED

Anikster Y, Eilam T, Manisterski J, Leonard KJ. 2003. Self-fertility and other distinguishing characteristics of a new morphotype of *Puccinia coronata* pathogenic on smooth brome grass. Mycologia 95:87–97.

———, Szabo LJ, Eilam T, Manisterski J, Koike ST, Bushnell WR. 2004. Morphology, life cycle biology, and DNA sequence analysis of rust fungi on garlic and chives from California. Phytopathology 94:569–577.

Berner DK, Bruckart WL. 2005. A decision tree for evaluation of exotic plant pathogens for classical biological control of introduced invasive weeds. Biol Contr 34:222–232.

Bruckart WL III, Eskandari FM, Becktell MC, Bean D, Littlefield J, Pilgeram AL, Sands DC, Aime MC. 2006. *Puccinia acroptili* on Russian Knapweed in Colorado, Montana and Wyoming. Plant Dis 90:971.

———, ———, Berner DK, Michael JM, Aime MC. 2005. A new aggressive rust fungus from Turkey is a candidate for biological control of Russian knapweed. Phytopathology 95(6):S14.

Cummins GB. 1979. Annotated, illustrated, host index of Sonoran Desert rust fungi. Mycotaxon 10:1–20.

DiTomaso JM, Healy EA. 2007. Weeds of California and other western states 1: Aizoaceae- Fabaceae. Publication 3488. Oakland, California: Univ. Calif. Agriculture Natural Resources.

Dugan FM, Carris LM. 1992. *Puccinia jaceae* and *P. acroptili* on knapweeds in Washington. Plant Dis 76:972.

Hildago O, Garcia-Jacas N, Garntje T, Susanna A. 2006. Phylogeny of *Rhaponticum* (Asteraceae, Cardueae-Centaureinae) and related genera inferred from nuclear and chloroplast DNA sequence data: taxonomic and biogeographic implications. Ann Bot 97:705–714.

Hiratsuka Y, Cummins GB. 1963. Morphology of the spermogonia of the rust fungi. Mycologia 55:487–507.

Kim WK, Mortensen K. 1986. Differentiation of *Puccinia jaceae, P. centaureae, P. acroptili*, and *P. carthami* by two-dimensional polypeptide mapping. Can J Plant Pathol 8:223–240.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth and Bisby's Dictionary of the Fungi. 9th ed. Oxon, UK: CABI Publishing. 650 p.

Mortensen K, Harris P, Kim WK. 1991. Host ranges of *Puccinia jaceae, P. acroptili* and *P. carthami* and the potential value of *P. jaceae* as a biological control agent for diffuse knapweed (*Centaurea diffusa*) in North America. Can J Plant Pathol 13:71–80.

———, Molloy MM. 1989. Fungi detected on *Acroptilon repens* (Russian knapweed) during surveys from 1981 to 1988. Can Plant Dis Surv 69:143–145.

Palm ME, Vesper SG. 1991. Russian knapweed rust caused by *Puccinia acroptili* in New Mexico. Plant Dis 75: 1075.

Savile DBO. 1970a. Some Eurasian *Puccinia* species attacking Cardueae. Can J Bot 48:1553–1566.

———. 1970b. Autoecious *Puccinia* species attacking Cardueae in North America. Can J Bot 48:1567–1584.

Sydow P, Sydow H. 1904. Monographia Uredinearum. Vol. 1. Lipsiae: Fratres Borntraeger. 972 p.

USDA NRCS. 2008, PLANTS Database (http://plants.usda.gov, 3 Apr 2008). National Plant Data Center, Baton Rouge, LA 70874-4490.

Wei SX, Wang YC. 1986. Taxonomic studies of *Puccinia* on Compositae in China. Acta Mycol Sin (Suppl. 1):185–226.